Importance of Lipids for Queen Fecundity and Colony Growth of *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

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ABSTRACT The importance of lipids for queen fecundity and colony growth of *Coptotermes formosanus* Shiraki was studied. Groups of 100 incipient colonies of *C. formosanus* were reared on artificial diet containing nine different soy lecithin concentrations. Eggs were counted every 15 d for a 5-mo period at 27 ± 1°C, 93 ± 5% RH, and 0:24 h (L:D) photoperiod. Fecundity per queen was estimated using a developmental rate-based graphic integration technique. At the end of a 1-yr period, the progeny of each colony was counted and recorded. Analysis of variance showed significant differences in queen fecundity and hatched progeny in colonies raised on various diets. Single linear regression analysis showed a small but significant linear increase in queen fecundity and hatched progeny per colony with increasing lecithin concentration. Increase in lecithin concentration explained ≈2, 4, and 8% of the increase in queen fecundity of colonies surviving 6 mo and 1 yr and number of workers and soldiers in 1 yr-old colonies, respectively. This indicates that, although intake of lipids increases queen fecundity and colony growth of *C. formosanus*, other factors not measured in this study alone or in combination with lecithin play major roles.

KEY WORDS Formosan subterranean termite, incipient colonies, nutrition, artificial diet, lecithin

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, feeds almost exclusively on cellulose materials. Some physiological and biochemical aspects of *C. formosanus* nutrition such as cellulose digestion and assimilation have been well studied. Cellulose is broken down by symbiotic protozoan fauna including the flagellates *Pseudotrichonympha grassii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi, inhabiting the termite proctodaeum (Lai et al. 1983, Yoshimura et al. 1992).

Cellulose molecules are broken into monosaccharides including glucose, galactose, arabinose, mannose, and xylose (Itakura et al. 1995). These monosaccharides are further broken down into acetic acid by anaerobic fermentation (Hungate 1943). Mauldin et al. (1972) reported the ability of *C. formosanus* to synthesize fatty acids, including oleic and linoleic acids, from acetic acid.

Although wood is an abundant resource, it may be deficient in some key nutrients, such as nitrogen, essential for the synthesis of proteins needed for insect development and reproduction. Symbiotic bacteria living in the hind gut of *C. formosanus* (Breznak et al. 1973) and other *Coptotermes* spp. (French et al. 1976) have been found to be capable of fixing nitrogen into nutritional components. One of these species living in the hind gut of *C. formosanus* was identified as *Enterobacter agglomerans* (Beijerinck) (Potrikus and Breznak 1977).

Nutritional impact on development of *C. formosanus* nutrition has not been well studied. Termites, being eusocial insects, display characteristics analogous to those of a superorganism (Wilson 1971). Reproduction is accomplished by a small reproductive caste, foraging is executed by a nonreproductive worker castes, and the brood and reproductive caste depend on workers for their nutrition, which is facilitated by trophallaxis (Wilson 1975). Within-colony population dynamics defines colony growth where birth rates are a function of the queen fecundity and death rates a function of worker longevity (Carey 1993). This is analogous to the growth, not reproduction, of a superorganism. Social insect demography is defined by the dynamics of colonies and their reproduction as they reach swarming size (Carey 1993). Studies on population biology of termites are more effective when directed to populations of colonies rather than populations of individuals.

Morales-Ramos and Rojas (2003a, 2005a) studied the development and nutrition of *C. formosanus* using incipient colonies. These studies showed that *C. formosanus* colonies feeding on different wood species develop at different rates and have different probabilities of long-term survival (Morales-Ramos and Rojas 2003a). Colony nutrition and foraging preferences are partially independent processes in *C. formosanus*

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that are not always explicable in terms of optimal foraging strategy of termite workers (Morales-Ramos and Rojas 2005a).

Differences in queen fecundity, colony growth, and survival of termites when feeding on different wood species may not be dependent exclusively on nutritional value of the wood. Besides cellulose and lignin, wood contains substantial quantities of other organic compounds including terpenoids, sterols, fatty acids, and carbohydrates (Buchanan 1963). In pine and spruce wood, the predominant fatty acids include oleic and linoleic acids, followed by palmitic and steric acids. Linoleic acid is predominant in aspen and birch wood with a much smaller proportion of oleic acid (Buchanan 1963). The complexity of wood chemistry makes it difficult to make inferences concerning termite nutrition based on differences observed in diets of different wood species. Growth and reproduction may be inhibited by toxic effects of secondary wood compounds obscuring observations of the effects of nutrition constituents. The use of artificial diets could eliminate this undesirable complexity by providing a more controlled experimental approach. The objective of this study was to determine the effect of variable lipid concentrations, administered in an artificial diet, on queen fecundity and colony growth of *C. formosanus.*

Materials and Methods

Incipient colonies of *C. formosanus* were initiated using methods described by Morales-Ramos and Rojas (2003a). Alates of *C. formosanus* were captured with a UV light trap (BioQuip Products, Rancho Dominguez, CA) located within the Southern Regional Research Center campus near New Orleans City Park during May and June 2003. Dealated males and females were paired in the laboratory by allowing them to walk on the surface of a paper towel inside 11.3-liter plastic boxes (Rubbermaid, Wooster, OH). Dealate pairs were collected by gentle aspiration, placed inside plastic vials, and provided with a wet piece of paper towel (Skilcraft; Industrial paper towel; National Industries for the Blind, Alexandria, VA) as described by Morales-Ramos and Rojas (2003a). Alate collection and pairing was done daily until 1,000 pairs were obtained. Vials containing pairs were kept at 27 ± 1°C, 93 ± 5% RH, and 0:24 h (L:D) photoperiod for a period of 2 wk. At the end of this period, those pairs that had oviposited were selected for the experiment.

A total of 900 ovipositing pairs were selected and transferred to small tight-fit dishes (50 by 9 mm) (Falcon Brand, No. 351006; Fisher, Pittsburgh, PA) and provided with 1 g of diet. Diet was formulated according Morales-Ramos and Rojas (2005b) and consisted of 33.54% alpha cellulose (Bio-Serv 3425, Bio-Serv; Frenchtown, NJ), 66.14% water, 0.08% ergosterol (Sigma E-6510; Sigma-Aldrich, San Louis, MO), and 50 ppm of solvent blue 58 colorant {9,10 anthracenedione,1,4-bis[(2-ethylexyl) amino], CAS No. 29887-08-9; Product 62418; Pylam Products Co., Tempe, AZ} (blue 50). The colorant was added for microbial control to reduce early incipient colony mortality (Morales-Ramos and Rojas 2005b). Different concentrations of soy lecithin (USB 18240; United States Biochemical, Cleveland, OH) were added to different treatment groups. There were eight treatment groups and a control group. Treatment groups were provided with 300, 600, 900, 1,200, 1,500, 1,800, 2,100, and 2,400 ppm of soy lecithin as part of the diet formulation. The control group was provided with a diet formulation deprived of soy lecithin. Each treatment group consisted of 100 incipient *C. formosanus* colonies.

All incipient *C. formosanus* colonies were kept in an environmental chamber at the conditions described above. Eggs were counted every 15 d for a period of 5 mo by direct observation under stereo microscope without opening the dishes. The shape and size of the dishes allowed direct observation of colony members without obstruction most of the time. In rare cases, when the brood chamber was completely covered, a gentle twist of the dish lid allowed the opening of the brood chamber without having to open the dish. The dishes were opened at the end of 1 yr to count and record the total number of workers (third instar or older), soldiers, and larvae (first and second instars) of each colony. These data were used to measure colony growth.

The measure of queen fecundity was the total number of eggs oviposited per queen during their first oviposition period, which extends to ~4 mo after colony foundation (King and Spink 1974). This was estimated from the biweekly egg counts for the first 5 mo after colony foundation using the graphic integration method of Kiritani and Nakasuji (1967) modified by Manly (1976) as adapted by Morales-Ramos and Rojas (2003a). This method maximized data collection of the first oviposition period without mixing data from the second oviposition period, which started ≈8.5 mo after colony foundation (King and Spink 1974). Only queens of those colonies that survived to the end of the 5-mo period were evaluated for fecundity and included in the analysis. Colonies that had lost the king before the end of this period were also eliminated from the analysis. This was done to ensure that evaluations of queen fecundity represented equal periods of oviposition time by fertilized queens among treatments. The effect of diet treatments on colony survival included all colonies as explained below.

Means of queen fecundity during the first 5 mo and mean number of hatched progeny at the end of 12 mo were analyzed by linear regression to determine the responses of these two variables to changes in soy lecithin concentration in the diet using JMP software (SAS Institute 2002). Survival of incipient colonies was measured by calculating the proportion of colonies with a surviving queen and king at a given age. Colonies without queen or king or any reproductive were considered to be doomed and recorded as dead colonies. Survival of incipient colonies among the different treatments was compared using a χ^2 test. The Z-test for categorical data (Ott 1984) was used to compare pairs of treatments.

Table 1. Mean eggs oviposited per queen during the first 5 mo after colony foundation and mean no. workers and soldiers in 1-yr-old colonies feeding in artificial diets with different concentrations of lecithin

Lecithin concentration	N	Eggs per queen[a]	n	Eggs per queen[b]	Workers and soldiers per colony
0	76	33.20 ± 17.62	42	39.35 ± 14.47	12.38 ± 8.45
300	70	36.00 ± 18.19	36	47.56 ± 14.46	14.89 ± 8.26
600	41	38.25 ± 16.11	26	46.05 ± 11.59	21.00 ± 9.78
900	62	36.05 ± 22.66	29	47.00 ± 22.28	16.59 ± 12.94
1,200	75	43.57 ± 29.46	35	52.41 ± 22.21	17.51 ± 10.49
1,500	70	38.71 ± 23.31	29	48.98 ± 21.28	19.14 ± 12.10
1,800	76	42.64 ± 24.66	43	54.68 ± 21.77	21.02 ± 8.51
2,100	74	45.63 ± 24.68	39	57.02 ± 20.23	23.69 ± 12.74
2,400	71	42.00 ± 24.54	38	49.39 ± 21.14	21.47 ± 13.30

[a] From colonies surviving 5 mo.
[b] From colonies surviving 1 yr.

Results

Means of number of eggs oviposited per queen were consistently higher in colonies surviving 1 yr compared with means calculated at the end of 6 mo, which included colonies that did not survive to the end of 1-yr period. Queens of colonies that survived to the end of 1 yr oviposited significantly more eggs (49.31) than queens belonging to colonies that died during the second half of the 1-yr period ($F = 57.08$; df = 1,608; $P < 0.0001$; Table 1).

A significant positive relationship was obtained between lecithin concentration in the diet and queen fecundity of colonies surviving 6 mo ($F = 13.19$; df = 1,613; $P = 0.0003$; $Y = 34.43 + 0.00428X$). Similarly, significant, positive relationships were observed between queens from colonies surviving 1 yr ($F = 14.01$; df = 1,315; $P = 0.0002$; $Y = 43.12 + 0.00505X$) and number of workers and soldiers in 1-yr-old colonies ($F = 25.89$; df = 1,315; $P < 0.0001$; $Y = 13.89 + 0.00384X$). Coefficients of determination (r^2) for these three regressions were low (0.021, 0.042, and 0.076, respectively), but hypotheses tests showed that the slope for all three models was significantly different than 0 ($T = 3.63$; df = 613; $P = 0.0003$, $T = 3.74$; df = 315; $P < 0.0001$, and $T = 5.09$; df = 315; $P < 0.0001$, respectively). This indicates that, although increasing dietary lecithin concentration can increase queen fecundity and colony growth, most of the variability in queen fecundity and colony growth results from the effects of unmeasured factors unrelated or partially related to lipid nutrition (Myers 1986). A better linear fit was obtained when means were analyzed instead of raw data: $r^2 = 0.698$ ($F = 16.16$; df = 1,7; $P = 0.0057$) and $r^2 = 0.739$ ($F = 19.78$; df = 1,7; $P = 0.003$) for regressions between lecithin concentration versus eggs per queen and versus workers and soldiers per 1-yr-old colony, respectively (Fig. 1).

Differences in colony survival among treatments were statistically significant only during the first month after colony foundation ($\chi^2 = 66.44$, df = 8, $P < 0.001$). Z-test revealed a significantly lower survival in colonies of treatment 600 compared with the rest of the treatments during the first month after colony foundation ($Z > 3.67$, $\alpha = 0.05$). No other significant differences were observed during this period. From the first month to the end of the experiment at 1 yr after colony foundation, no significant differences in colony survival were observed (Fig. 2).

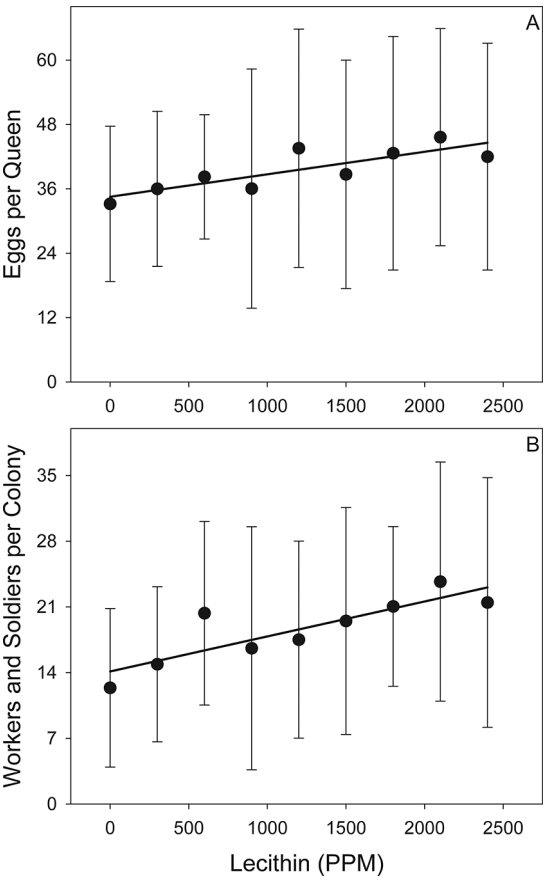

Fig. 1. Regressions between means of queen fecundity of colonies surviving 1 yr and lecithin concentration in ppm (A) ($Y = 34.53 + 0.0042X$) and between mean number of workers and soldiers per 1-yr-old colony and lecithin concentration in ppm (B) ($Y = 14.13 + 0.0037X$). Brackets represent SD.

Discussion

Results from the regression analysis showed strong evidence that increases in lecithin concentrations significantly increased queen fecundity and colony growth of *C. formosanus.* The effects of lecithin concentrations on queen fecundity and colony growth, however, were extremely subtle, with slope values of only 0.0013 and 0.00075, respectively, and coefficient of determination values indicate that a variation of lecithin concentrations explain a small percentage of the variation of queen fecundity and colony growth. However, these effects indicate that lipid nutrition is an important factor in *C. formosanus* colony growth. This study did not find significant effects of lipid diet on colony survival. The only significant differences observed were in the treatment containing 600 ppm of lecithin during the first month

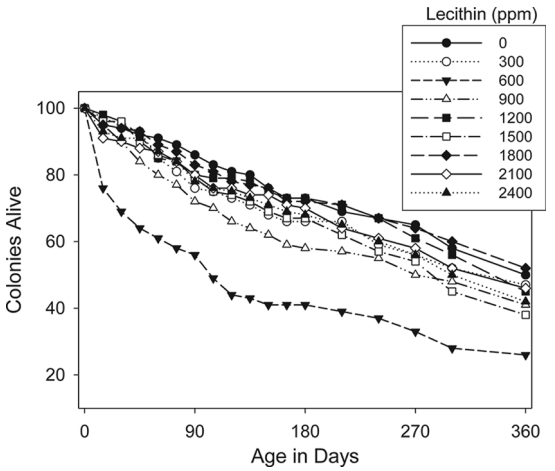

Fig. 2. Survival of *C. formosanus* colonies feeding on diets with different concentrations of lecithin.

after colony foundation (Fig. 2). Because the highest mortality occurred in a treatment with an intermediate lecithin concentration, these differences in colony mortality probably were not caused by the treatment effect.

Fatty acid content varies substantially in different wood species (Morales-Ramos and Rojas 2003b), and wood species significantly impacts colony growth and survival of *C. formosanus* (Morales-Ramos and Rojas 2003a). This study suggested the possibility that at least some of the effects of wood species on colony growth may be explained by differences in lipid content among different wood species. This study was not designed to identify specific essential fatty acids for reproduction and growth of *C. formosanus.* Lecithin is a complex mix of many lipids including palmitic, linoleic, oleic steric, and linolenic acids (Merck 2001). Oleic, palmitic, linoleic, and steric acids are commonly found in wood, and their ratios and concentrations vary among the different species (Buchanan 1963). If these lipids are present in wood and they provide nutritional benefits to termite colonies, they may play an important role in termite food selection.

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